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Propagation of Selected Native Prairie Plants by Leaf Tissue ulture

Bernard Erlyn Ellis

PROPAGATION OF SELECTED NATIVE PRAIRIE PLANTS BY LEAF TISSUE CULTURE

BY

BERNARD ERLYN ELLIS

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PRAIRIE PLANTS BY LEAF TISSUE CULTURE

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable of meeting the thesis requirements for this degree. Acceptance for this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Botany-Biology Department Date

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INTRODUCTION

Preservation of our native prairie has long been neglected while extensive farming practices have reduced the native prairie to isolated areas of untillable land. These diminishing acres of prairie (Jenkins 1971, Tinker 1971) are our remaining link with the stable environment after man has disrupted the landscape to his advantage.

In an abandoned wasteland, nature has the power to heal by ecological succession. Succession begins not only where man has scarred the landscape, but also in the path of floods, fires, glaciers, and other natural forces. It is a basic ecological principle that succession starts with an early colonization of a few species specialized for soil stabilization and the accumulation of organic matter. In the next stage of succession, different and diverse combinations of species use the minerals and materials produced by the earlier fewer species and soon cover the ground and displace them. Increasing diversity, stability, and order are the results of succession (Odum 1971).

Succession can only begin on disrupted land if the great diversity of prairie plants are available to provide a seed source. Unless we make a special effort to preserve these complex communities, they will be forfeited to poor land use planning. This loss will result in a weakening of the health and the stability of the ecological systems on which we depend (Humke 1975).

Concern over our environment is bringing about the realization that once the prairie disappears, its gene pool that developed over thousands of years will be irretrievably lost. For years, scientists have been advocating the preservation of plant genetic resources, but the warnings have gone largely unheeded (Miller 1973). For example, developing new varieties of wheat is increasing yields, but it is also resulting in the loss and modification of genes. This causes a narrowing of the genetic base, and characteristics with no apparent value are lost. These warnings apply, not only to food crops like wheat, but to all plant species. Unless the habitat to support this gene pool is maintained, species will become extinct and whatever value they may have possessed will be permanently extinguished (Humke 1975).

The aesthetic value of these native prairie plants is not to be overlooked. The variety of colors, lack of maintenance after they are established, hardiness, and natural adaptability to this climate make them ideal for use in highway rights-of-way, industrial parks and gardens. In addition, re-establishment of prairie has potential for historic sites, farm yards, church yards, study areas, and flower gardens (Stevens 1973, Lofgren 1973, Mayer 1971).

In order to rescue prairie plants from extinction, the propagation and multiplication of prairie plants have become important. Responses of many plant genera to tissue culture have been examined but previous examination of prairie plant responses are limited

(Chen and Holden 1975). Vegetative propagation methods, seed viability and germination studies, and environmental needs of prairie plants have been studied (Sorenson and Holden 1974, Blake 1935, Schramm 1970, Lawrence et al. 1947, Greene and Curtis 1950). The problems that are pointed out in these conventional propagation techniques include poor seed germination, lack of roots on stem cuttings, difficulties in harvesting viable seed, securing enough seed to work with, lack of seed set, or destroying the desirable individual, especially an endangered species, through a root or stem cutting. These problems may be circumvented through the use of tissue culture.

Almost any organ of a plant can be selected as an explant for a callus source (Murashige 1974), but utilizing the leaf as an explant source (Raju and Mann 1970, Sun 1966, Rao et al. 1973, Bristow 1962, Chlyah 1973, Kohlenbach 1965, Steeves et al. 1957) offers certain advantages. If the plant is an endangered species, using a few leaves for propagation does not ruin the plant as root or stem cuttings could. Also, leaves are much easier to secure in the field. Using very young healthy leaves also insures one of cells that are still actively dividing and growing, and they are less likely to be diseased than older stem or root explants.

When compared to seedlings grown in the field, tissue cultured plantlets produced are healthy, large and vigorous, weed competition is overcome (Mayer 1971) and the result is usually earlier blooming of the tissue cultured material.

Gene preservation is another advantage in that these tissue

culture plants are produced from diploid plant cells and are identical to their parents (Steward et al. 1964) and all the genes of the parent plant are maintained. It is also useful to use the technique of tissue culture on unique prairie plants which are occasionally found in the field. These sports, if containing a desirable characteristic, can be multiplied many times by culture. If left, they would probably die out because of failure to set seed, or they would be reabsorbed by the dominant genetic races.

Tissue culture material can also be used as a genetic bank by storage of callus (Frankel 1974). It can be used in liquid shake culture to produce plantlets (Steward et al. 1952). Shake cultures can also produce protoplasts needed to conduct experiments in the production of new and different genetic combinations that will not normally happen by hybridization methods (Gamborg 1974). Recently these protoplasts have been induced to pick up genes by transduction (Shanmugan and Valentine 1975).

The production of plantlets in tissue culture usually follows two patterns. First, the formation of adventive shoots and roots through organogenesis, or secondly, formation of adventive embryos called embryogenesis (Murashige 1974). The process of organogenesis usually proceeds mainly after the formation of a small lump of tissue which has dedifferentiated from the original explant. Differentiation then occurs to roots, shoots, or other organs depending on the hormonal concentrations and ratios used, morphological location of the explant source, and the differentiating tissues (Gautheret 1966).

Embryogenesis usually begins at the upper surface of the callus from a cell which, after forming a multicellular clump, initiates the totipotent embryo (Chen and Holden 1973). After initiation of an embryo, it develops in much the same way as in a seed. Hormonal concentrations and ratios also influence the development of embryos from the callus.

Determining whether plantlets produced are via embryogenesis or organogenesis lies in microscopic examination of the structures involved. Classifying a plant structure as an embryo is based on its bipolarity and that at its earliest developmental stage it has at opposite ends a shoot and a radicle. In the case of monopolar buds and roots, it is always possible to show their connection with the vascular elements in the callus (Street 1973).

The objective of this investigation was to produce plantlets of selected native prairie plants by leaf tissue culture and to eventually establish them in the field. In doing this it was necessary to study the characteristics of each species in both culture and field behavior. Twenty species were selected for study in the investigation (Table I). Special attention is given to the value of this technique for propagation of rare and endangered species and unique phenotypes that may prove valuable in maintaining a broad genetic base for future use.

MATERIALS AND METHODS

The commonly accepted methods of tissue culture employing Murashige and Skoog (M&S) mineral medium (Murashige and Skoog 1962) and using young leaves as explants (Gupta et al. 1966, Kasperbauer and Collins 1972, Maheshwari et al. 1965) were employed to determine the use of the technique for cloning prairie plants.

Two commonly used hormonal ratios (Skoog and Miller 1957) were selected as a means to screen prairie plants that would respond to tissue culture. Napthaleneacetic acid (NAA) was substituted for indoleacetic acid (IAA) in the Murashige and Skoog media. These ratios were almost the reciprocal of each other with medium I having a ratio of 30 parts kinetin (K) to 1 part NAA (3 ppm K : 0.1 ppm NAA), and medium II having a ratio of 1 K to 20 NAA (0.1 ppm K : 2 ppm NAA). Only those plants that responded favorably to one or the other of the above media were selected for further study. In this manner 20 common prairie plants were screened and cultured during the growing season of 1974.

The first method was to culture field collected leaves during the growing season. Young healthy leaves were obtained from the Sioux Prairie, 20 miles south of Brookings, South Dakota, on old highway 77 (T107 N, R50 W). The leaves were brought back to the lab in moist chambers, sterilized and placed on the media.

The second more successful method was to germinate seeds and grow seedlings in the laboratory as a source of leaf explants.

Table I: The Responses of Leaf Tissues to Various Media

Species	Common Name	Medium[d]	Response
Achillea millefolium ssp. lanulosa[a]	Western Yarrow	I	Leaf curl[c]
		II	Brown callus
Anemone patens[a]	Pasque Flower	I	No response
		II	Brown callus
		2,4-D CM	No response
Artemisia frigida[a]	Prairie Sagewort	I	Leaf curl
		II	Roots
		2,4-D CM	Callus
Asclepias tuberosa[b]	Butterfly Milkweed	I	Embryos
		II	Callus
		II CM	Callus
Cicuta maculata[b]	Water Hemlock	I	Leaf curl only
		II	No response
Echinacea angustifolia[a]	Purple Coneflower	I	Shoots, roots
		II	Callus
Geum triflorum[a]	Torch Flower	I	No response
		II	No response
Liatris spicata[a]	Dotted Gay-Feather	I	Callus, roots
		II	Callus, roots
		10 K	Callus, roots
		30 K	Callus, roots

Table I, continued

Species	Common Name	Medium	Response
Oenothera biennis[a]	Evening Primrose	I	Brown callus
		II	Roots
Pedicularis canadensis[a]	Common Lousewort	I	Leaf curl
		II	Brown callus, roots
		2,4-D CM	Callus
Penstemon grandiflorus[b]	Shell-Leaf Penstemon	I	Leaf curl
		II	Callus, roots
		2,4-D CM	Callus
Phlox pilosa[a]	Downy Phlox	I	Shoots
		II	Roots
		II CM	Callus
Ratibida columnifera[a]	Yellow Prairie Coneflower	I	Shoots
		II	Callus, roots
Ratibida columnifera fa. pulcherima[a]	Purple Prairie Coneflower	I	Shoots
		II	Callus, roots
Rudbeckia serotina[b]	Brown-eyed Susan	I	Shoots
		II	Roots
Senecio aureus[a]	Golden Groundsel	I	Leaf curl
		II	No response
Solidago rigida[b]	Golden Rod	I	Leaf curl
		II	Callus, roots
		2,4-D CM	Callus

Table I, continued

Species	Common Name	Medium	Response
Viola nuttallii[a]	Nuttall Violet	I	Leaf curl
		II	Callus, brown
Viola pedatifida[a]	Prairie Violet	I	Leaf curl
		II	Callus, brown
Zizia aptera[a]	Golden Alexander	I	Callus
		II	Embryo

[a] Field collected leaves were the source of explants.

[b] Laboratory grown seedling leaves were the source of explants.

[c] A typical leaf curl response is shown in Fig. 1a.

[d] CM indicates that 10% coconut milk was added to the medium.

In all cases the leaves were sterilized for 10-20 minutes in a constantly stirred, 2% filtered solution of calcium hypochlorite with the addition of a wetting agent, Tween 20. From there, they were passed through three changes of sterile distilled water and then placed on the media. All transfers were carried out aseptically in a hood under positive air pressure.

Leaf explants and subsequent callus were kept in a 25°C dark growth chamber. After signs of differentiation appeared, the bottles were placed in weak-diffuse light at 25°C for continued development.

After roots, shoots, or embryos had developed on hormonal nutrient media, these plantlets were placed on a lowered hormone concentration of 0.1 ppm K and 0.1 ppm NAA to promote normal root and shoot growth. Following this, they were placed on M&S mineral medium with sucrose but minus growth regulators to further enhance root formation. When sufficient root growth had developed, they were placed under fluorescent light (800 Fc with a 16 hour daylength) in vermiculite. Beakers were placed over the new plants to cut down on transpiration. Gradually they were conditioned by placing larger containers over them until they could stand normal room temperature, light, and humidity levels. After being conditioned to normal room environment, a small fan was used to circulate the air around the plants in order to eliminate damping-off. While in vermiculite, plantlets were fed with one-half strength Hoagland's solution. Later, they were transferred to pots and placed in a greenhouse environment or transplanted to the field.

Tissue cultured specimens were killed in formalin acetic alcohol (FAA) and dehydrated in the tertiary butyl ethanol series. They were embedded in paraffin and the 10µm sections were stained with safranin, crystal violet, fast green and orange G, cleared in clove oil and mounted in Harleco synthetic resin.

On those species which did not produce plantlets on either medium I nor medium II, hormone concentrations were varied from the described procedure as shown in Table I. In one instance, (A. patens) plant parts other than leaves were also tested to evoke a response.

RESULTS

Response of the prairie plants to leaf tissue culture is shown in Table I. Of the twenty species tested, only three had essentially no response. Five species responded with callus, but either it was hard, very slow growing, and dark brown, or it had a high concentration of anthocyanin. Differentiation via organogenesis occurred in ten species. Normal callus and adventive roots appeared on five of these species, but no adventive shoots occurred. Callus of the other five species formed adventive shoots on medium I and roots on medium II. Differentiation via embryogenesis occurred in two species. Callus of one species differentiated into embryos on medium I and the second species callus showed embryo formation on medium II.

In the plant species which differentiated through organogenesis, production of adventive roots and shoots occurred. R. columnifera exemplified how leaf explants produced plantlets. Very young healthy leaves from the field were collected. Best results were obtained when leaf length was less than 3 centimeters. After sterilization, the leaf was placed in bottles (Fig. 1a) on medium I and medium II. After 3-4 weeks, callus had developed (Fig. 1b) on medium II. It was then transferred to medium I where shoots initiated (Fig. 2a) after 2-3 weeks. If the differentiating shoots were left on medium I, roots eventually appeared (Fig. 2b). Microscopic examination (Fig. 3) revealed that these meristematic growing points were

monopolar and attached to vascular elements in the callus or the original explant source (Sass 1958). After roots occurred, the small plantlet was transferred to a lowered (0.1 ppm K and 0.1 ppm NAA) or zero hormone on M&S mineral medium for 2 weeks for root development before placing it in vermiculite. After acclimatization, plantlets appeared as in Fig. 4a before they were placed in pots to induce flowering (Fig. 4b) or set out in the field (Fig. 5). The above procedure was followed with the remaining prairie plants. Field collected leaves which were very difficult to sterilize were replaced with leaves from seedlings grown in the laboratory.

R. columnifera fa. pulcherima, while following the above pattern, took about six weeks longer to differentiate roots after shoots had appeared. Both sets of explants had identical media and environmental conditions, and were selected at the time of flowering from field collected leaves.

Older callus from both field collected and laboratory grown leaves on medium I was the source of differentiation of E. angustifolia. The callus had a hard texture and was brown in color when shoots appeared. After 4-6 weeks on a 0.1 ppm K and 0.1 ppm NAA M&S mineral medium, roots differentiated and the callus was subsequently transferred to vermiculite (Fig. 6a). This plant generated a large number of shoots and very broad leaves prior to transplanting to the field.

Organogenesis on medium I occurred very rapidly with leaf

explants of R. serotina. From inoculation of a laboratory seedling leaf until removal from the flask to vermiculite was 6-8 weeks. The large number of shoots and rapid growth in vermiculite (Fig. 6b) resulted in very large leaves.

Profuse root formation occurred with field collected leaves on medium II with P. pilosa. Shoot differentiation only occurred on medium I and then it was transferred to medium II to stimulate root formation. Shoot elongation and growth was slow after differentiation did occur.

Embryogenesis accounted for two of the differentiating prairie plant explants, one of which is shown in Fig. 7. A. tuberosa seedling leaf explants immediately began to form callus on medium II. When this medium was supplemented with 10% coconut milk, increased callus growth occurred. Upon transfer from medium II to medium I, embryos began to differentiate in 10-14 days (Fig. 8). When shoot length approached 3 centimeters, they were transferred to M&S mineral medium supplemented with 3% sucrose. After the shoots had grown to approximately 5-10 centimeters, (Fig. 8b) the plants seemed to go dormant. Roots never adequately developed, and subsequent transferring to vermiculite did not stimulate growth. Since A. tuberosa favors slightly alkaline soil, pH on the zero hormonal M&S mineral medium with sucrose (which usually had a pH range of 5.5-5.8) was adjusted to a neutral 7.0 or a slightly basic pH of 7.1-7.2. This had no stimulatory effect on root growth. Limited elongation of the shoots did occur.

Embryo formation also occurred with Z. aptera. Field collected leaves callused readily on medium I and upon transfer to medium II, roots appeared. No shoots were initiated from these roots. But after 2 months on medium II, embryos began to form and the response was similar to the formation of carrot embryos (Chen and Holden 1973, Steward et al. 1952).

G. triflorum had no response to either medium I or II, while only on medium I did C. maculata and S. aureus show a curling of the leaf, which turned white. No callus, adventive roots or shoots, or embryo formation appeared. Eventually the leaf explants which did not curl turned brown and apparently died. Transferring the curled leaf from medium I to medium II did not evoke any response.

O. biennis showed a brown callus formation on medium I. No shoots or roots occurred on this medium but transfer to medium II induced root formation.

A. millefolium, V. nuttallii, and V. pedatifida on medium I did cause the leaves to curl, but no shoot or root differentiation occurred. On medium II a very slow growing, hard, brown callus formed. No differentiation occurred and subsequent transfers to medium I did not induce differentiation or stimulate callus growth.

A. patens was also a very difficult species to handle. Only in one instance, on medium II, did callus form but it was brown and very slow growing. There also was no response to 2,4-dichlorophenoxyacetic acid (2,4-D) with 10% coconut milk. Different plant parts were also tried, to include ovary, stamen, anther, pedicel,

and a young root; all eventually turned brown and no other response was noted.

A. frigida, P. canadensis, P. grandiflorus, and S. rigida showed a curling of the leaf on medium I, but no callus formation or adventive differentiation to roots, or shoots occurred. Leaf explants placed on medium II showed subsequent callus formation. After the callus was 4-6 weeks old, adventive roots occurred from the callus. Supplementing medium II with 10% coconut milk or using 2,4-D with 10% coconut milk only stimulated callus growth. Transfer of this callus to medium I induced no differentiation.

L. spicata readily formed friable, rapidly growing callus on both medium I and II. However, only root differentiation could be induced. Even when kinetin concentrations were raised to 30 ppm, no shoot differentiation occurred, only increased callus growth and the appearance of adventive roots.

Growth of tissue cultured plantlets in the field compared to those appearing on the Sioux Prairie is illustrated by Fig. 9 and Fig. 10.

Fig. 9 shows a striking example between blooming of a tissue cultured R. columnifera transplanted in May, 1974 (Fig. 9a) and one which was transplanted to the field in May, 1975 (Fig. 9b). Note the larger number of flower stalks and the increased vigor of the transplanted tissue cultured R. columnifera plantlets as opposed to the R. columnifera found on the Sioux Prairie (Fig. 9c).

Tissue cultured R. serotinas (Fig. 10a) are contrasted with those found on the Sioux Prairie (Fig. 10b). Though the flowers are arising from 3 tissue cultured plantlets in Fig. 10a, note their superior performance to those appearing in the field.

DISCUSSION

Selection of the media for screening the prairie plants was based on the two general types of media which have been found to induce differentiation; medium I which is essentially the tobacco medium of Skoog and Miller (1957), and medium II, essentially Steward's (1952) carrot medium. These workers found that root and shoot initiation is basically regulated by interactions between auxin and cytokinin.

Those species responding to the tobacco medium essentially in the same manner as tobacco explants were R. columnifera, E. angustifolia, R. columnifera fa. pulcherima, and P. pilosa. In these cases the development was through organogenesis with kinetin influencing shoot formation and auxin influencing root formation. Kinetin was essential for organogenesis, although auxin could be eliminated and roots would eventually occur spontaneously if shoots were allowed to grow.

Z. aptera, which responded to the carrot medium, behaved much as carrot leaf explants both in organogenesis and embryogenesis, but with the latter predominating. In this case the most essential hormone was auxin but kinetin was not dispensible. In contrast, A. tuberosa produced embryos only on the tobacco medium where there was a high concentration of kinetin.

In those unresponsive species some undiscovered factor(s) is still missing. Additional research is needed to discover conditions

that may cause these species to respond. The three primary factors to investigate are variations in K : NAA balance, other hormones, and environmental conditions. Other more specific factors involved are alternation of temperature, selection of an explant at the proper physiological age, light, and the physical quality of the medium.

In those prairie plants which flower very early in the Spring (A. patens), regulation of the temperature to stimulate callus growth may be very critical. While these cultures were maintained at a constant 25°C, this does not take into consideration the normal temperature fluctuations of a plant in the field. Numerous studies point out that an optimal shoot or root initiation temperature may be other than 25°C and alternation of temperature may also be essential.

Selection of explants at the right physiological age may overcome some of the problems involved. Almost any organ or tissue can be used as an explant source, but each species needs to be thoroughly investigated to determine which explant source responds. Some species regenerate best with leaves and other species do not respond with leaves at all (Heide 1965).

The physiological age and the amount of differentiation among neighboring cells may account for differences in meristematic initiation. It has been found that in flowering plants of tobacco that explants selected near the base produce vegetative buds while explants selected near inflorescences produce flowers (Chouard and Aghion 1961). Raju and Mann (1970) found that in Echeveria elegans

explants, using young leaves produced only roots, older leaves initiated shoots, and leaves intermediate between the two produced both roots and shoots.

Organogenesis and embryogenesis can be affected by light. The factors of quality, daylength, and intensity influence shoot and root initiation and asexual embryo initiation (Nebel and Naylor 1968, Gautheret 1969, Haccius and Lakshmanan 1965). Considering the wide range of photoperiodicity of the prairie plants selected, light probably influences differentiation responses in a number of them.

The final factor is the physical quality of the medium, i.e. whether it is a liquid medium or a solid medium. The change from liquid to solid media influences shoot initiation, cell and asexual embryo development and vegetative vs. flower bud regeneration (Skoog 1944, Ammirato and Steward 1971, Sun 1966).

Establishment into the field proved to be difficult with only one species investigated, A. tuberosa. In this case, dormancy or lack of vernalization occurred in plants grown from seeds as well as plantlets produced in culture. The plants produced 10-15 leaves and then stopped growing even when placed on a 16 hour daylength in the laboratory. When transplanted to the field in the middle of May, these dormant plants still failed to grow.

From repeated trials it was found that leaves from field collected material were much more prone to contamination than laboratory grown seedling leaves. Collecting leaves from the field usually resulted in more than 90% contamination, while leaves

selected from seedlings grown in the laboratory became contaminated less than 10% of the time.

Establishment of callus is a major problem in tissue culture. If the callus is slow growing, it takes time to establish a sufficient amount of callus to work with. If the contamination rate is low, then many leaves can be successfully innoculated at once and time is saved. To cut down on the contamination rate, soaking the field collected leaves in Hoagland's solution for 12-24 hours may help by providing conditions for growth of spores lodged in leaf cracks and spaces. It is much easier to kill the actively growing fungi than the dormant spores. Therefore, as a matter of practical need, considerable care must be given to developing a suitable source of explants in order to keep the contamination rate low.

Finally, the ease by which these prairie plants grew in pots until flowering suggests using them for house plants. R. serotina, R. columnifera, and E. angustifolia callused readily from leaf explants obtained from laboratory grown seedlings. Once differentiation occurred, shoots and roots developed quickly. Growth in vermiculite was rapid and they transplanted well to soil. After initial flowering, they can be cut back, fertilized, and if kept in a long day condition, will continue to flower. The hardiness and lack of care needed for prairie plants could make them ideal for use in the home.

CONCLUSION

Propagation of native prairie plants by tissue culture was successful. The value of this technique lies not only in preserving a broad genetic base, but also in preserving unique combinations of characters possible only through cloning.

Seven of the 20 species studied produced plantlets. The ease by which these plantlets were produced are rated in the following order: R. serotina, R. columnifera, E. angustifolia, R. columnifera fa. pulcherima, A. tuberosa, P. pilosa, and Z. aptera. Four of these were established in the field and were more vigorous than their field counterparts.

Success in producing plantlets with medium I (high K) was greater than with medium II (high NAA) with 6 species responding to the former and only 1 to the latter. Four of the species produced plantlets via organogenesis and 2 species via embryogenesis.

One species, A. tuberosa, may require vernalization while in the callus stage or prior to establishing them in vermiculite to overcome dormancy conditions.

Five species produced good friable callus and adventive roots, but no shoots were ever noticed. Additional work spent on these species may result in plantlets being produced.

Eight of the species were either non-responsive or produced atypical growth patterns. Altering environmental conditions or changing the morphological location of the explant source may result

in the successful establishment of these species.

Using leaves from seedlings grown in the laboratory was more successful than leaves selected from the field. The contamination rate on leaves from the field was over 90% while it was less than 10% for laboratory grown seedlings.

Using this technique to propagate endangered species would be particularly valuable. It would also be desirable for genetic variations found in the field, as in the range of colors (from dark purple to cream) found in L. spicata or the yellow to red petals in R. columnifera to R. columnifera fa. pulcherima.

This investigation shows that while propagation of prairie plants by tissue culture is successful, additional work is needed. This work establishes some of the guidelines whereby routine cloning of a species may be possible.

FIGURES

Fig. 1. Procedure used to initiate R. columnifera leaf tissue cal

A. Leaf curl response prior to callus initiation

B. Friable callus produced from leaf tissue on medium II

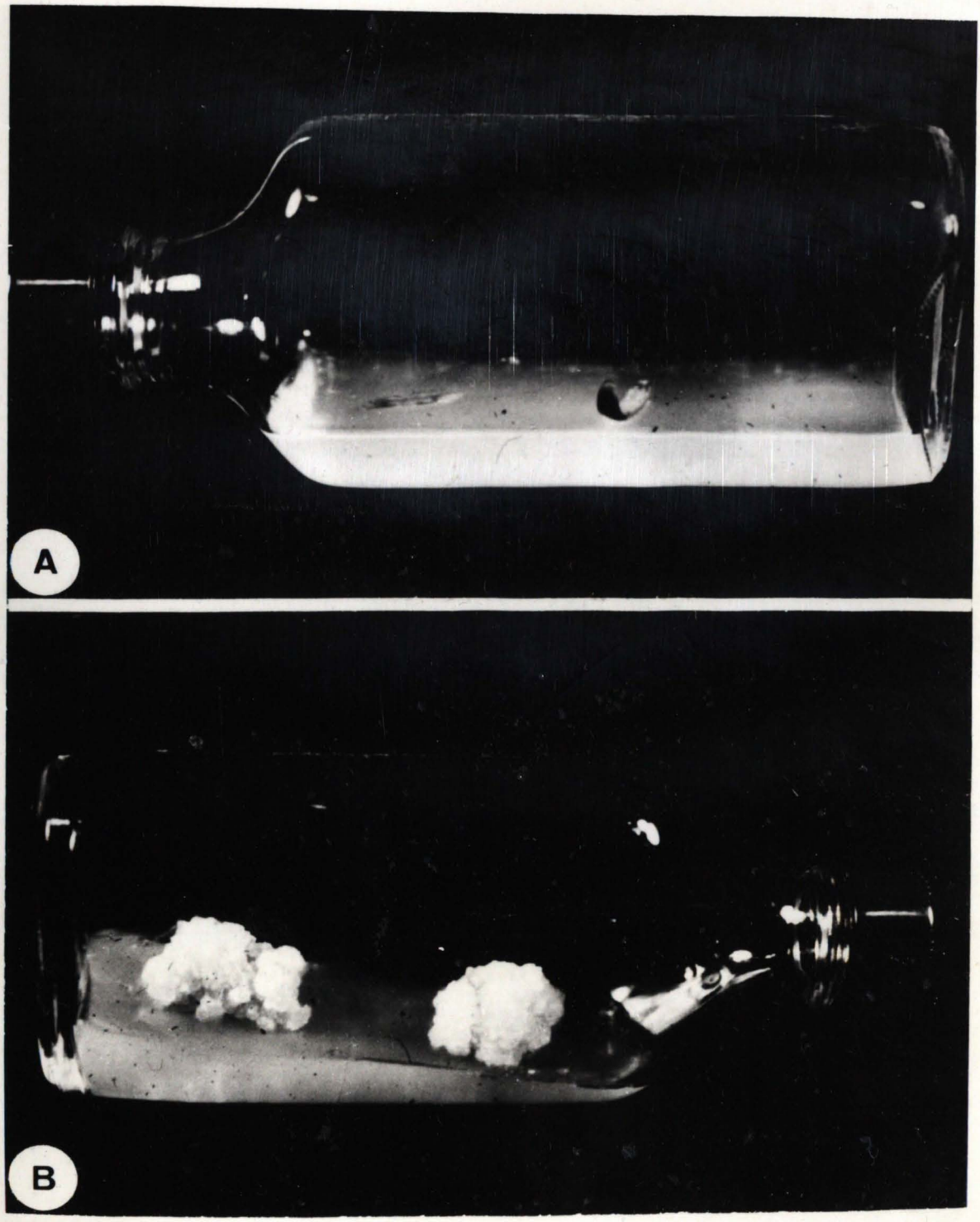
A
B

Fig. 2. Effect of media on differentiation of callus tissue from R. columnifera

A. Shoot formation on medium I

B. Root formation on medium II

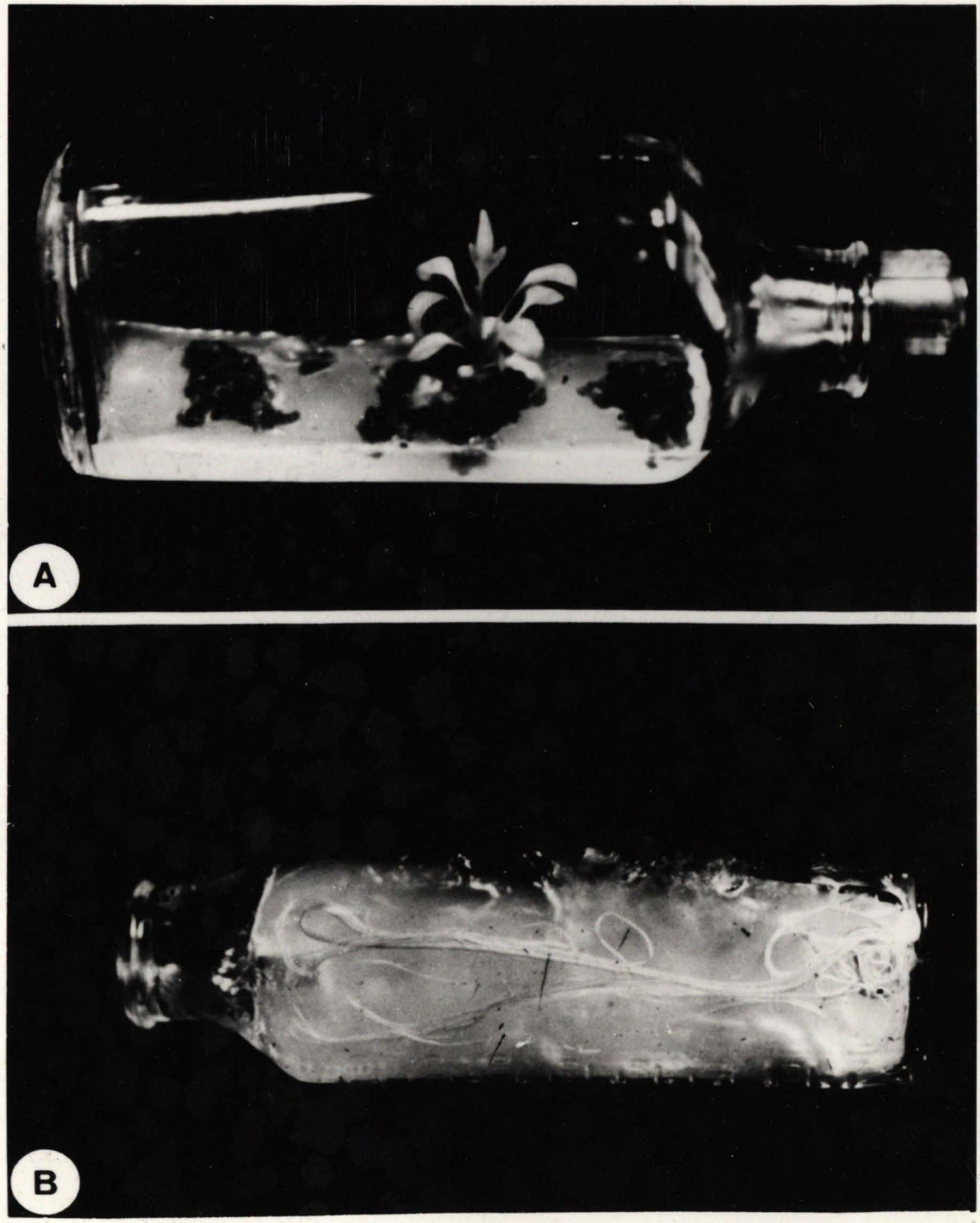
A
B

Fig. 3. *In vitro* organogenesis from R. columnifera callus Unlabeled arrowhead indicates parenchyma cells in callus tissue and unlabeled arrow indicates meristematic region in callus. Note leaf primordia (L) surrounding the growing point

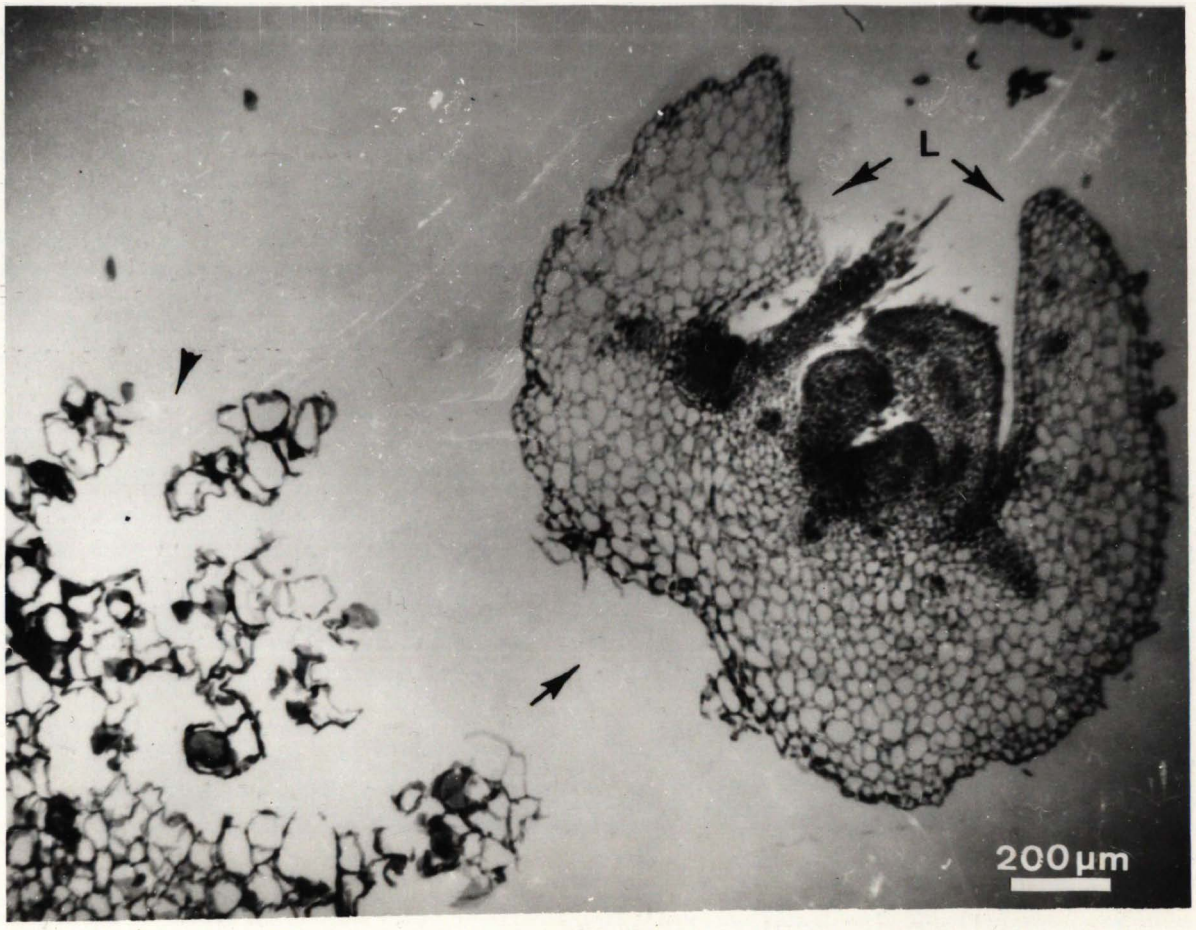
L
200µm

Fig. 4. Transplanting of *In vitro* plantlets to vermiculite and soil

A. *In vitro* R. columnifera plantlets established in vermiculite

B. Flowering R. columnifera potted in soil

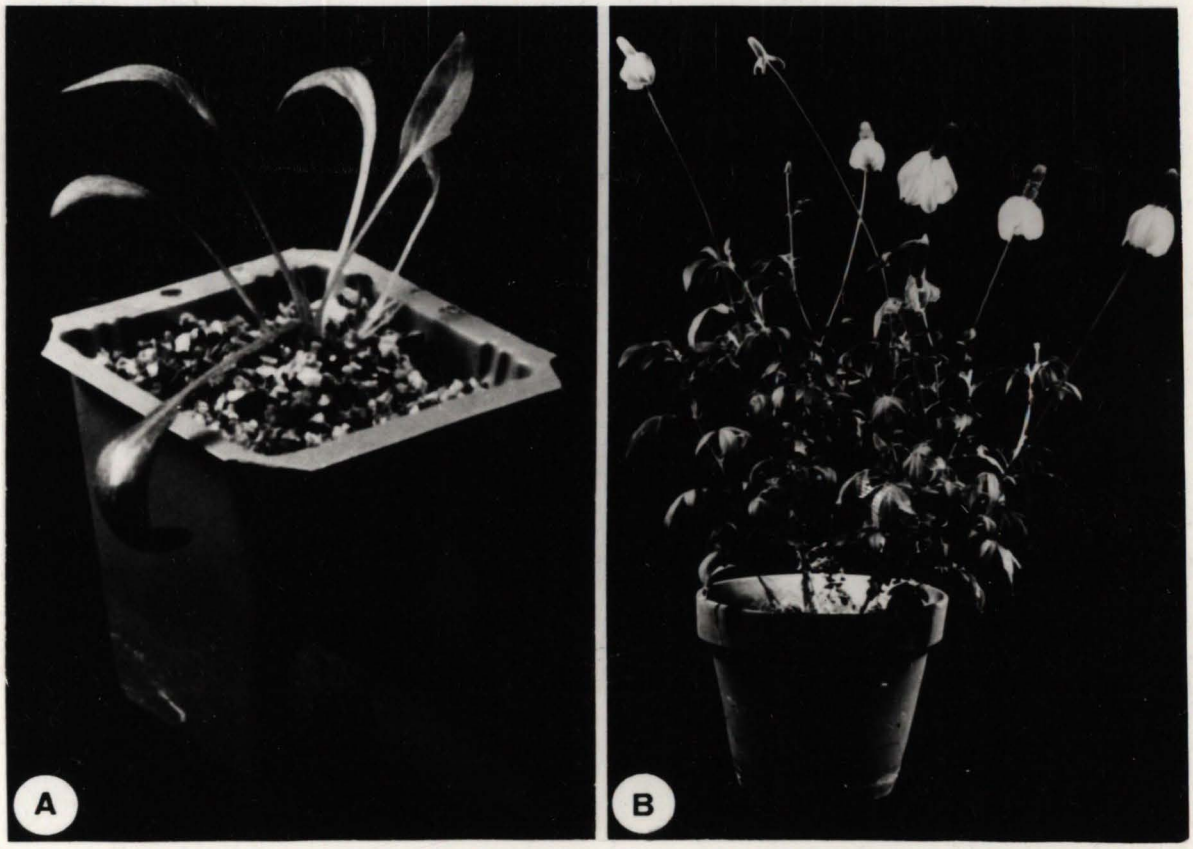
A
B

Fig. 5. *In vitro* R. columnifera established in the field

Fig. 6. *In vitro* plantlets established in vermiculite

A. E. angustifolia

B. R. serotina

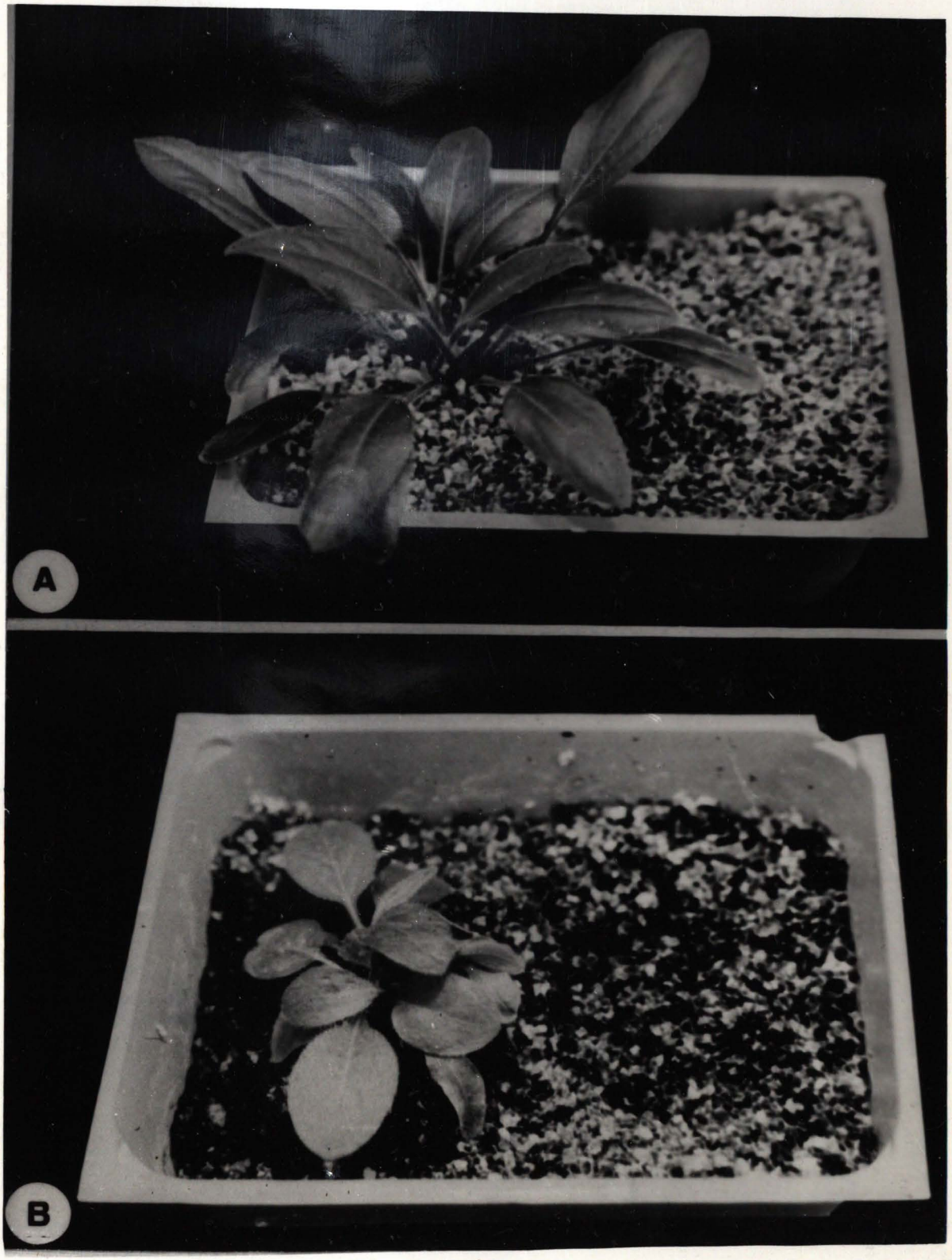
A
B

Fig. 7. 10µm section of A. tuberosa embryo produced on medium I


500 µm

Fig. 8. Embryogenesis in A. tuberosa

A. A. tuberosa embryos in various stages of development

B. Plantlets grown from embryos similar to those in Fig. 8a

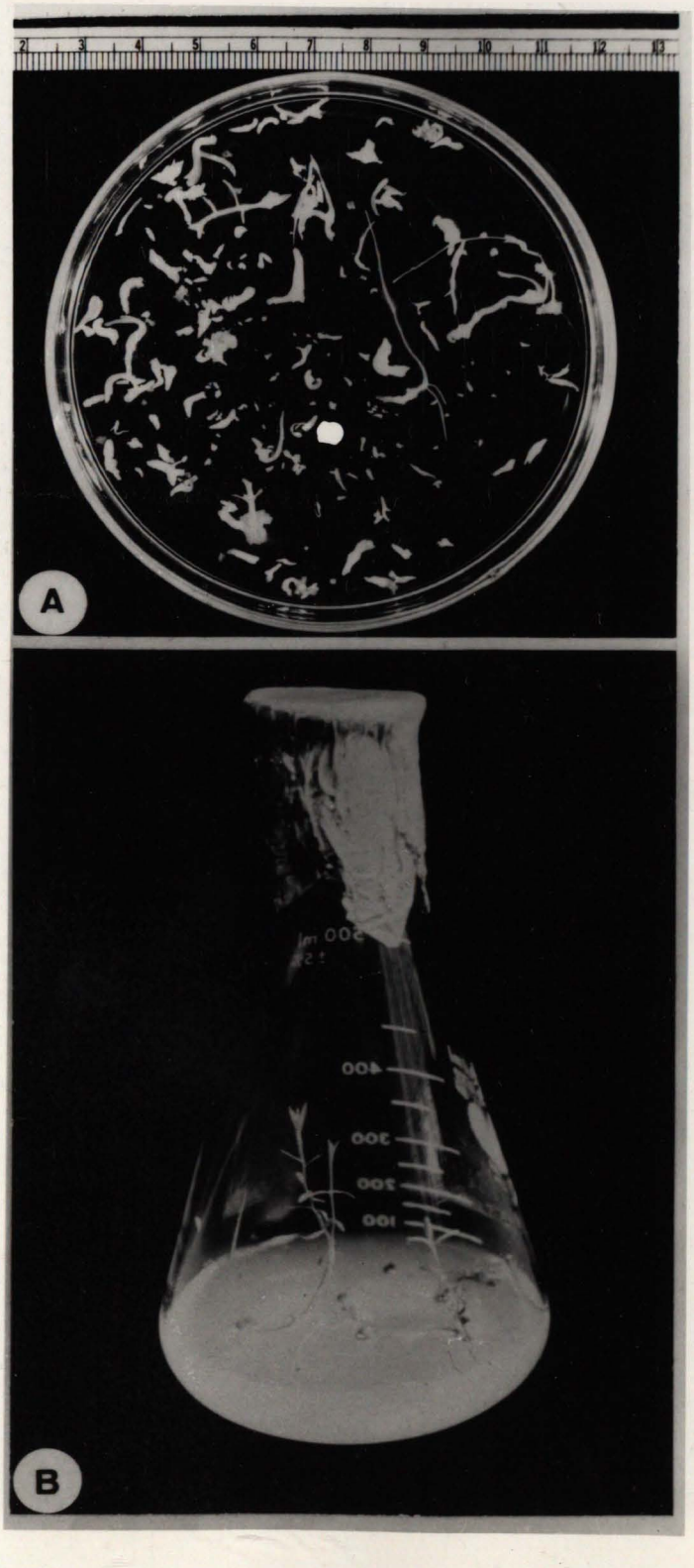
A
B

Fig. 9. R. columnifera plants

A. Tissue cultured R. columnifera plantlet transplanted to the field in May, 1974

B. Tissue cultured R. columnifera plantlets transplanted to the field in May, 1975

C. R. columnifera plant appearing on the Sioux Prairie


A
B
C

Fig. 10. R. serotina plants

A. Tissue cultured R. serotina plantlets transplanted in May, 1975

B. R. serotina appearing on Sioux Prairie

Note the increased vigor of tissue cultured plantlet in Fig. 10a


A
B

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